Plant Archives Vol. 19, Supplement 1, 2019 pp. 1206-1213 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

PHOTO BLEACHING INHIBITION ACTIVITY OF *CUCURBITA PEPO* FRUIT EXTRACT ON BRILLIANT BLUE DYE

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Abstract

In the present study, methanolic extract of the *Cucurbita pepo* fruit plant was prepared and tested for the anti-oxidantactivity using DPPH. The results showed that the methanol extract works as an antioxidant and has been underwent to chemical analysis. The analysis showed that it contains most popular plant components except saponiens and steroids .The industrial dye Brilliant Blue was prepared with a concentration $1x10^{-4}$, irradiated in a photolysis cell in the presence of the methanolic abstract and TiO_2 catalyst. The results showed that the absolute removal rate of dye was 50.85% in the absence of the catalyst and after two hours of irradiation with UV, while the absolute removal rate of dye was 98.39% with the presence of catalyst ,antioxidant methanol extract, of hexane, chloroform, ethylacetate, 1-butanol. To elucidatte the activity on the dye photo bleaching further extraction of methanolic extract with 0.1 g were added to the cell containing the dye with concentration $1x10^{-4}$ and irradiated. It was noticed that the dye survived degradation with the presence of antioxidant and this shows the role of the extract protecting the dyes photo bleaching when exposed to UV light. The results showed that among the most effective extracts on the dye was ethyl acetate extract compared to other extracts compounds and then the effective compound was isolated characterized by IR, H-NMR, Mass spectra, the effective compound was suggested to be(1-(4-tert-butylphenyl)4-[4-[hydroxydiphenyl methyl] piperidin-1-yl] butyl)1- butanol

Keywords: Methanol extract, photo degradation, *Cucurbita pepo,* Photo bleaching, dyes; UV-vis.

Introduction

UV light 280-400 nm is a major responsible of generation of free radicals by the mechanism of reactive oxygen species (ROS) involving the formation of O_2^-, O_2^1, H_2O_2, $^{\cdot}OH$, $ROO^{\cdot}$, etc.;(Herrling, Jung and Fuchs, 2007). these free radical generated by UV radiation can play a key role in the degradation of organic material (Burrows *et al.*, 2002). The molecules that inhibited or delayed the reactivity of free radical known ROS scavenging molecules, such as L-ascorbic acid (vitamin C) and α-tocopherol (vitamin E) (Herrling, Jung and Fuchs, 2007). in present study, *Cucurbita pepo* fruit extract was used to test the extract antioxidant reactivity versus the free radical generated by the UV light.

UV absorbers preferentially absorb the incident UV radiation and so protect the substratefrom the radiation. UV absorbers do not themselves degrade rapidly, but they convert UV energy into harmless levels of heat energy, which are dissipated throughout the substrate matrix. UV absorbers are limited in their effectiveness because of the physical limitations of the absorption process and their ability to absorb is governed by the need for high concentrations of additive and thickness of substrate before sufficient absorption will occur to retard the photo degradation effectively.

However, high concentrations of additives would be uneconomic and technically limited, while many applications are in very thin sections, such as film and fiber. Benzophenones are good general-purpose UV absorbers for clear polyolefin systems, and can also be used in pigmented compounds. Benzotriazoles are used mainly in polystyrene. Both can also be used in polyesters. Concentrations are usually about 0.25-1.0% The main function of UV absorbers is to absorb UV radiation in the presence of a chromosphere (Ch) found in the substrate, the aim is to filter out the UV light that is harmful to the substrate before Ch* has had a chance of forming. Above all, a UV absorber must function within the 290 and 350 nm range. During this process, the absorbed energy is converted into vibrational and rotational energy of the molecule constituents. For UV absorbers to be effective, it is essential that this process takes place more rapidly than the corresponding reaction within the substrate, and that neither the UV absorber nor the substract is intended to stabilize is damaged during energy conversion. The aim of the present study is to protect the BB dye from fading by the action of artificial UV light incorporating*Cucurbita pepo* fruit extract with the BB solution

Materials and Methods

Materials

Chemical Substances

(1,1-diphenyl—picrylhydrazyl DPPH (BDH England), Hexane (BDH England) 99%, Ethanol (BDH England) 99%, Methanol (BDH England) 99%, 1-butanol (BDH England) 99%, Chloroform (BDH England) 99%, Ethyl acetate (BDH England) 99%, ferric Chloride (BDH England) 99%, Hydrochloric acid 1%, Potassium iodide, lead acetatehydrate, Sodium Hydroxide, α- Naphthol, Copper sulphate, n-hexane, Sodium carbonate, Silica – Jel, Ammonia solution,

Instruments

Equipments: Rotary evaporator, Oven 2004 Japan – Hirayama, Ultrasonic bath (England), Uv-Visible Spectrophotometer, double + 90Plus (Japan, Vortex mixer, Hot plate stirrer, Drying Oven, 1H NMR Bruker 500 MHz, Water bath (Shimadzu), Mass spectrometer (Japan)MS Model: 5975C VL MSD with Tripe, IR Spectrophotometer FTIR affinity Spectrometer (Shimadzu) Japan

Experimental set-up

Extraction

Cucurbita pepo plant was collected from northern Iraq province of Arbil *Cucurbita pepo fruit* was cut into smooth pieces powdered it room temperature for a period of four days weighted 130gand extracted with 100 mL methanol/water mixtures 70/30 (vol/vol) extraction done by continuously stirring of crudes solution for 6 hours at 40 °C in Ultrasonic bath

The solvent was evaporated with the aid rotary evaporator to obtain dried crude methanolic extract. The methanolic extract was then suspended in distilled water in a separatory funnel and partitioned successively with hexane, chloroform, ethyl acetate, and 1-butanol to obtain fractions in these solvents. This process left residual aqueous fraction at the end. The solvents were removed with a rotary evaporator at low pressure (about 10 mbar) to produce dried fractions.

Preliminary qualitative phytochemical analysis

1. Tests for alkaloids : (Gayon, 1972).
2. Tests for tannins : (Gayon, 1972).
3. Tests for steroids :(Harbone, 1984).
4. Tests for flavonoids: (Harbone, 1984).
5. Tests for saponins : (Harbone, 1984).
6. Test for coumarins: (Harbone, 1984).
7. Tests for phenols: (Gayon, 1972).
8. Test for DPPH: (Curr. Med. Chem. 2007)

Photo bleaching:

Irradiation experiments were performed in a cylindrical photo reactor. The volume of Brilliant blue solution was 100m of $1x10^{-4}$M. The temperature controlled by water cycling and addition of ice cubes to water bath when the UV irradiation process cause temperature elevation of photo reactor

Known amount of extract was added to the dye solution (20 ppm) stirrered to the time required to reach equilibrium. After stirring, the solution was irradiated by UV illumination,. Sampling suddenly was 5 ml pipetted form the mixture after a period of time. The samples were separated by centrifuge (6000 rpm) for UV-Vis analysis. The Dye concentration estimated by UV-Vis spectroscopy at wavelength corresponding to maximum absorbance 602nm (λ max).

Separation of the components of the ethylacetate extract by thin chromatography

The components of the ethyl acetate extract(after methanol/water extract) of the *Cucurbita pepo fruit* were separated by TLC using the following systems; readymade plates of silica gel (20x20cm) of 0.25 mm thickness (MERCK)

solvent = Hexane : ethyl acetate (2:5) Solvent system was prepared and placed in a glass tank (22.5 cm x 22 cm x 7 cm) covered with a glass lid.

Results and Discussion

Methanol/water Extract of *Cucurbita pepo fruit* plant was extracted with hexane, chloroform, ethyl acetate, 1-butanol. Results were tabulated in table 4.1

Table 1 : Phytochemical test of *Cucurbita pepo* fruit extract

Compound	Detector	Detection Guide	Ethanol extract	Methanol Extract	Hexane Extract	Chloroform extract	Ethyl acetate extract	Butanol Extract
Alkaloides	Meyer	turbid	+	+	+	+	+	+
Phenols	$FeCl_3$	Bluish green color	–	+	–	–	+	+
Flavonoid	KoH + Ethanol	yellow color	–	+	–	–	+	+
Tannines	Lead acetate	White precipitate	+	+	–	–	+	+
Coumarin	UV	blue color	+	+	+	_	+	+
Glycosides	Molisch	Red deposit	+	+	+	+	+	+
Saponins	$HgCl_2$ (1%)	White precipitate	+	–	–	+	–	+
Steroid	H_2SO_4+ $CHCl_3$	Red deposit	–	–	–	–	–	–

Chemical presence(+) Chemical no presence(⁻)

Methanol extract from *Cucurbita pepo* fruit plant as antioxidant was measured by DPPH method results were presented in Fig. (1) total inhibition was 79.74% compared with that of ascorbic acid Fig. (2) which is 96%

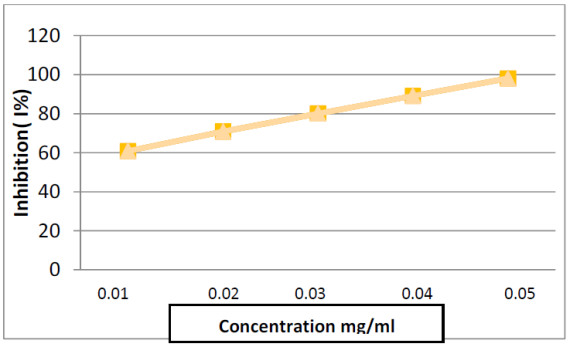

Fig. 1 : Methanol extract from the *Cucurbita pepo fruit* plant in DPPH free radical inhibition compared to; ascorbic acid

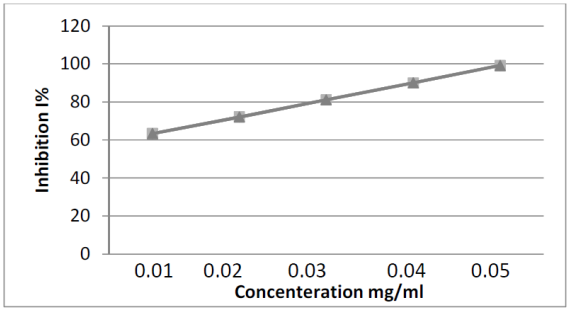

Fig. 2 : Ascorbic acid inhibition with DPPH free radical inhibition

Effect of extract addition on the decolarization of BB dye

The effect of different exracts (0.1 g) on the photo decolorization of 100mL of BB dye solution (20 ppm) was spectrophotometerly investigatied by using UV-Vis spectroscopy. The decolorization ratio of dye by UV illumination were 84.49%, 30.25%, 15.78%, 42.73 and 84.69 respectivley for methanolic, butanolic, ethyl acetate, hexane, and chloroform extracts without added TiO_2, respectivley (see fig.7). The absorption spectrums (200-1100 nm) of BB dye solutions before and during irradiation were shownin Fig. (3). the decrease in the decolorization of dye in precence of ethyl acetat extract inay to anti oxidant that inhibted the oxidation process on the molecule dye by neutralizing highly reactive hydroxyl radicals ($^{\bullet}OH$) and decreased the rate of radicals formation (Lü *et al.*, 2010).

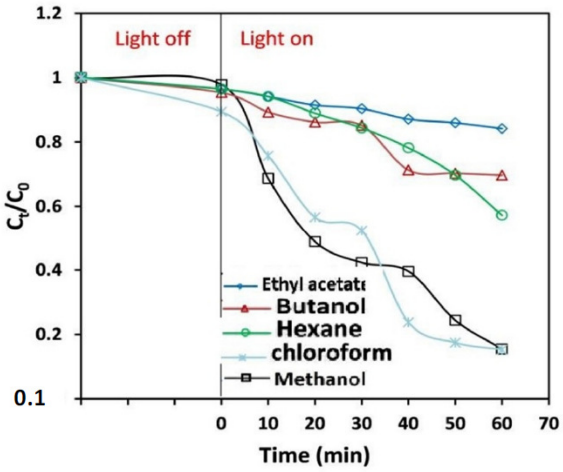

Fig. 3: the extract type effect on the photo decolonization of BB dye

Effect of extract mass on the decolorisation of BB dye

In order to evaluate the effect of extract mass on the decolorisation of BB dye, different amount were performed photo decolorisation. Other parameter kept constant, initial pH=7, initial concentration 20 ppm, and add weight (0.100g, 0.050g, 0.015g) of the methanol extract to the dye BB, so that the removal of the dye will decrease as illustrate in figure (4). Depending on the amount of anti-oxidant that present in the extract, the inhibition of photo degradation reaction will be more efficiency.

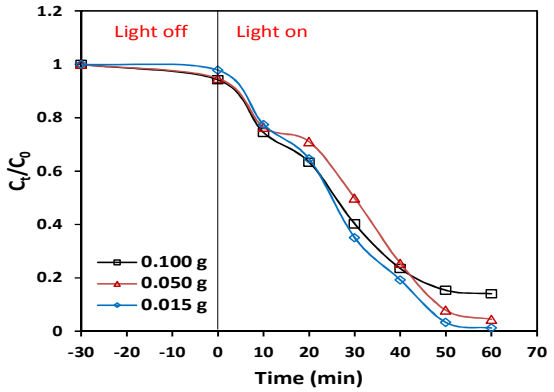

Fig 4 : The extract mass effect on the hotodecolorisation of BB dye

Initial concentrations effect on the decolorisation of BB dye

Initial concentration is important parameter applied to investigate the efficiency of photo decolorisation of the dye in water. Three different initial concentrations of dye solution 20 ppm used and other parameters kept constant, room temperature, pH 7, 0.1 g of methanol extract and 0.03 g of TiO_2 catalyst. The extent of decolorisation reduced from 96.12% to 85.91% as the initial concentration increase from 10 ppm to 20 ppm and as illustrate in figure5.the reason of such effect may attributed to the colour developed with increasing concentration of the dye(Kumar and Pandey, 2017).

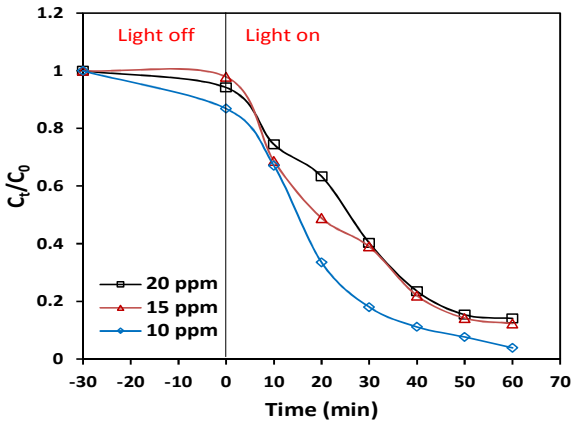

Fig. 5 : The initial BB dye concentrations effect on the photo decolorisation

Effect of catalyst on the decolorisation of BB dye

Fig (7) shows that the percentage of absolute removal of the dye was 49.15% without catalyst TiO_2 after two hours of Irradiation in photolysis Cell while the removal ratio dye 98.93% in presenceing TiO_2 which is equivalent to approximation Double the removal ratio in absence catalyst TiO_2

Precence of TiO_2 nanoparticles act as catalyst that imporved the rate of dissociation of water into highly reactive hydroxyl radicals (•OH). These formed •OH radicals is responsible to the attack on N=N bond in the dye sturcture causing color of dye fading. Whreseas, the decolorization of dye was decreased in presense of ethyl acetate extract because of the scavenging free radicals wich rhay, or prevent the oxidation of dye molecule (Santhosh Kumar, C.N.; Shridhar, N.B.; Jagadeesh, S.S.; Sanganal, J.S.; Suguna, R.; Ansar Kamran, 2014)

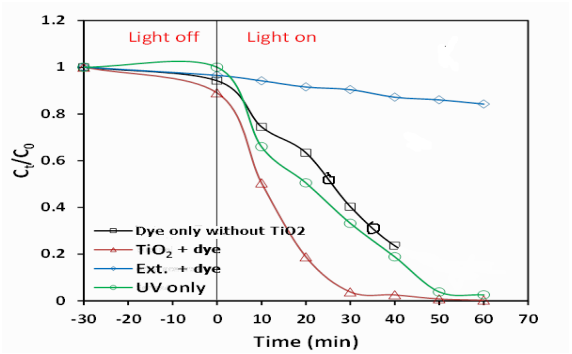

Fig. 6 : Catalyst effect on the photodecolorization of BB dye

Kinetic of the decolorisation of BB dye

The photo-degradation of dye in present of TiO_2, TiO_2 with extract, or only extract can be described successfully by kinetics of pseudo first-order (Eq.1) (Martínez Nieto *et al.*, 2009).

$$\ln\left|\left(\frac{C}{C_0}\right)\right| = -k_b t$$

where C_t and C_0 are the concentrations of dye at time t and time 0, respectively, and kb is the apparent first-order photo degradation rate constant.

All decolorisation data fit well to pseudo first-order kinetics by the plotting between the time and Ln (Ct/C_0) that used to estimate the linear equation as shown in Figure (9). The rate constant (k) of dye decolorisation and conditions were shown inable (2). presenc of TiO_2 nanoparticles causes increase in rate of decolorisation of dye, due to the activated generation of the free electrons and holes on the TiO_2 surface and generate hydroxyl free radicals when TiO_2 irradiated with UV or near UV light .

Fig. 6 : Extracts from primary methanolic extract were presented in figure 7.Ethyl acatate was the best extract prerent photofading of the dye

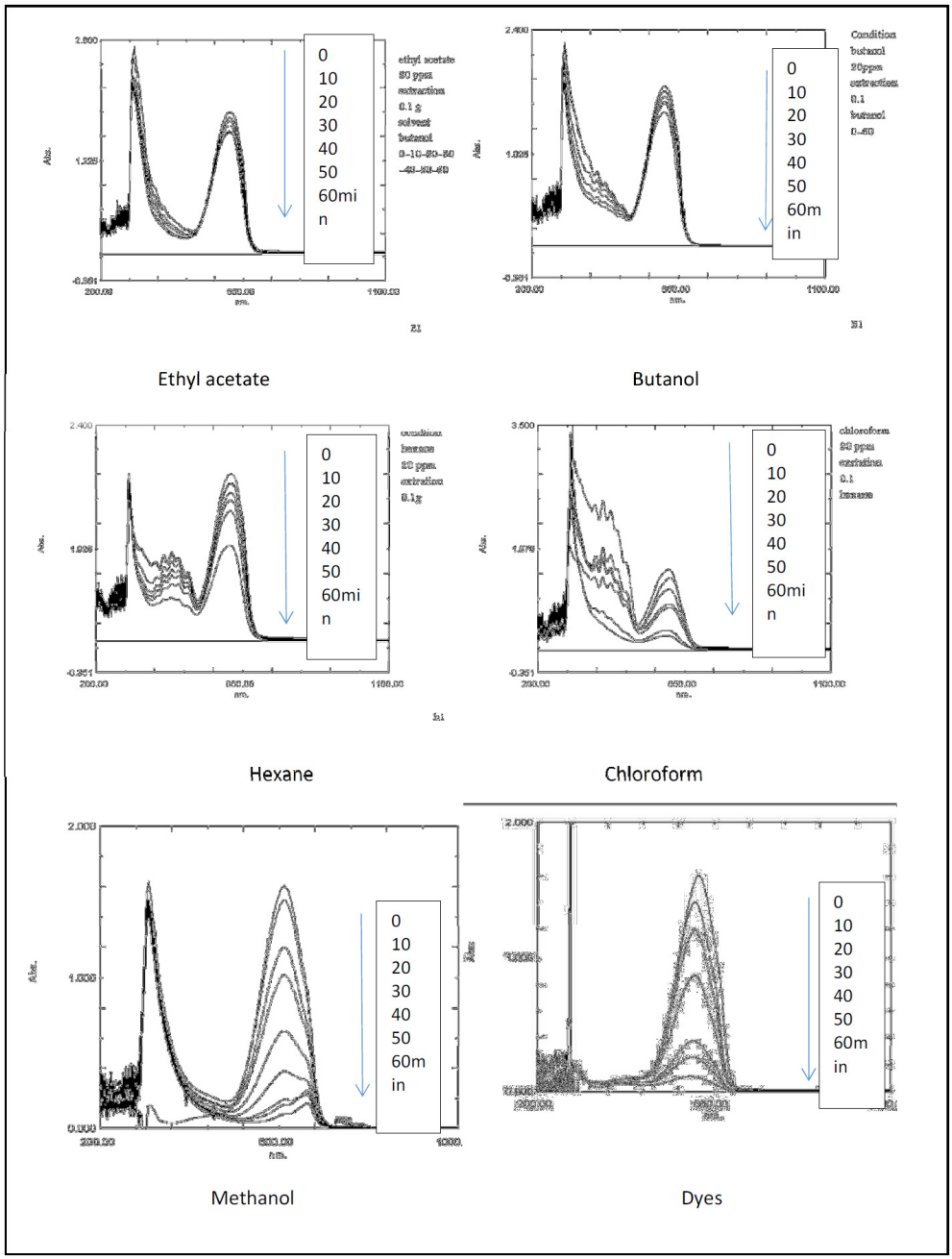

Fig. 7 : Decolirigation of BB dye by UV light in presence of different extracts

Table 2 : Determination of rate constants and correlation coefficients for the dissolution dye BB at different temperatures.

Conditions	Rate constant ($10^{-3}min^{-1}$)	r^2
In presence TiO_2	97.2	0.987
In presence TiO_2 + Ext.	35.2	0.970
In presence Ext.	2.3	0.990

These generated hydroxyl radicals were responsible to oxidize and degrade/ decompose organic dye 4.8: characterization and idrntification of isolated compound Ethyl acetate extract was Chromatographic, isolated, and characterized.

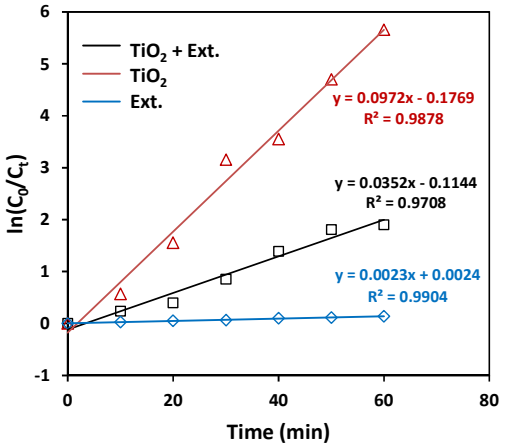

Fig. 8 : Dseudo first order plot of the dye photo decolorisation

The result of TLC examination is show in figure 9 compared with the result of the TLC extract fig 10 pmir to column separation the collected fraction were isolated and characterited by TLC .

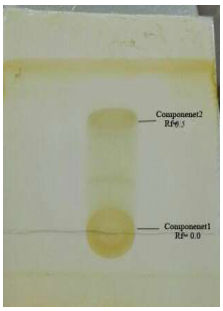

Fig. 9 : TLC after separation of isolated compound

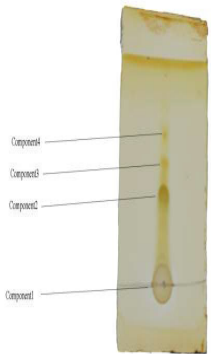

Fig. 10 : TLC Before the separation process

The important peaks observed in the IR spectrum of the isolated compound and the structural units inferred Figure 11 The hetroaromatic structure indicates that the presence of CH stretching vibration in the range 3000 – 3100 cm^{-1}, which is a characteristic region for three C-H stretching vibration The C=C ring stretching vibrations for all rings assigned to 1604.77 cm^{-1} in the flavone A peak at 1350 cm^{-1} in IR spectrum of isolated compound indicates the presence of C-N group The O-H group vibrations are likely to be the most sensitive to the environment, there for, they show pronounced shifts in the spectra of the hydrogen–bonded species. ′H-NMR shown in Figure (12).

The H-6 and H-8 resonance appear at δH 6.8 ppm and δH 6.9 ppm respectively, and they show meta – coupling. The two protons occur as a double the resonance arises as a result of presence of the four hydrogens on the B – ring. The H-2′ and H-6′ pair occur in identical environments, and they are centered at δH 7.79 ppm and 7.82 ppm. While the H-3′ and H-5′ pair, also occur in identical environments, appear as resonance centered at δH 7.4 ppm. Mass Spectrum for the isolated compound shown in Figure (13)the mother ion peak appears at m/z471 for compound of a formula. ($C_{32}H_{41}NO_2$) According to the about mentioned spectroscopic data the suggested structure of the compound in *Cucurbita pepo* fruit.

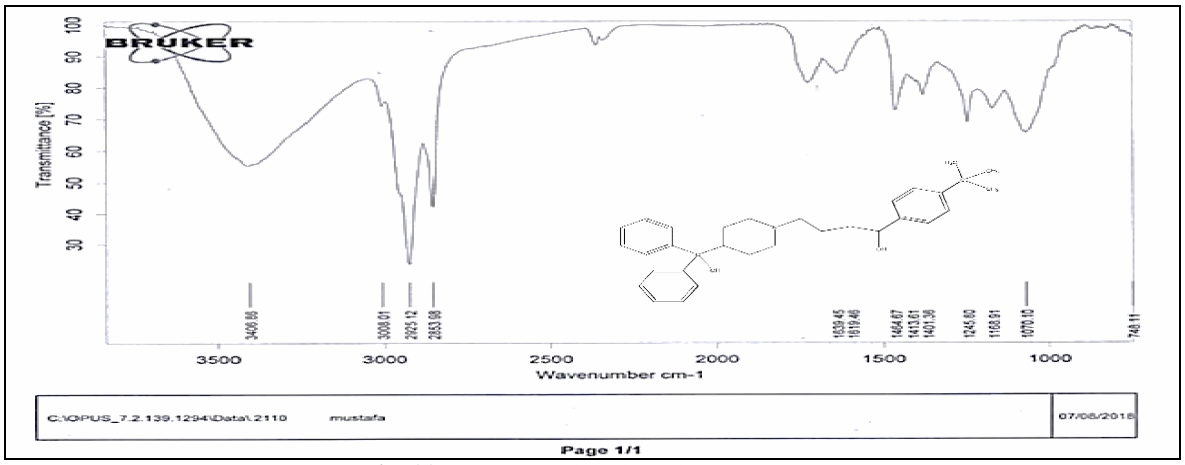

Fig. 11 : Spectrum of the isolation compound

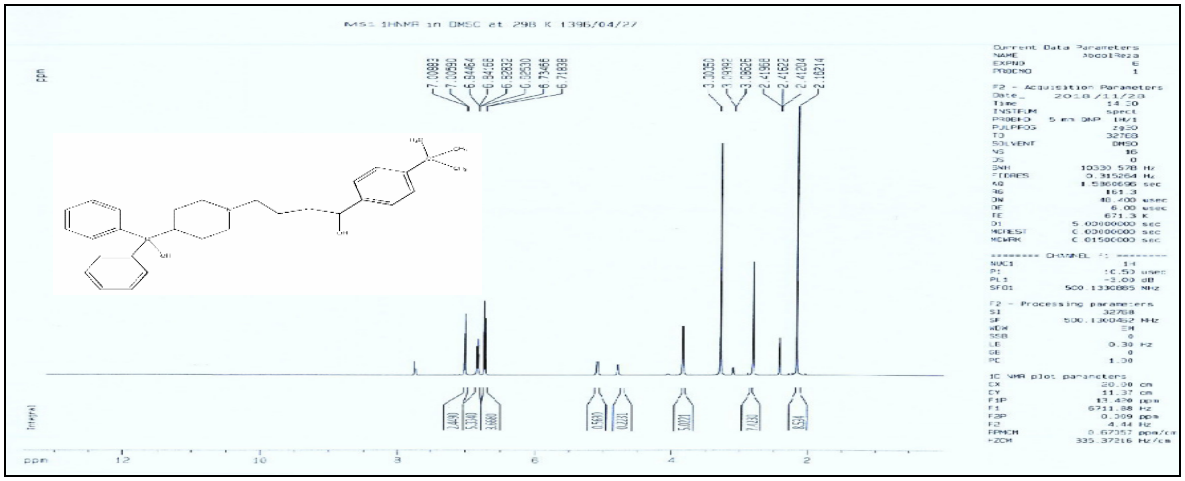

Fig. 12 : H-NMR spectrum of the isolation compound

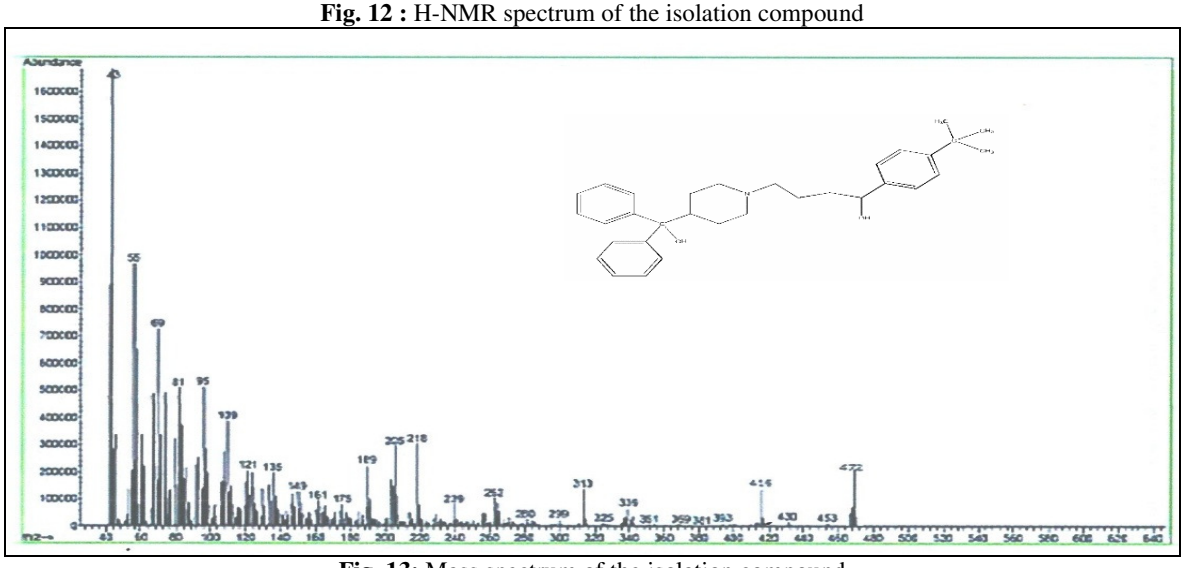

Fig. 13: Mass spectrum of the isolation compound

References

Burrows, H.D. *et al.* (2002). 'Reaction pathways and mechanisms of photodegradation of pesticides', *Journal of Photochemistry and Photobiology B: Biology*.

Dissanayake, D.M.R.H. (2018). 'The Study of Antioxidant and Antibacterial Properties of Skin, Seeds and Leaves of The Sri Lankan Variety of Pumpkin', *IOSR Journal Of Pharmacy*, 8(2), pp. 43–48.

Gautam, A. *et al.* (2016) 'Photodegradation of organic dyes based on anatase and rutile TiO_2 nanoparticles', *RSC Adv*. The Royal Society of Chemistry, 6(4), pp. 2746–2759.

Herrling, T.; Jung, K. and Fuchs, J. (2007). 'UV-Generated Free Radicals (FR) in Skin and Hair', *SÖFW-Journal* |, 133(8): 2–11.

Kapilan, R. (2015). 'Determination of Antibacterial Activity of Some spices', *International Journal of Research- Granthaalayah*, 3(10): 57–64.

Kumar, A. and Pandey, G. (2017). 'Preparation and Photocatalytic Activity of Co: La: Tio_2 Nanocomposites for the Degradation of Methyl Blue in Visible Light', *JOJ Material Sci*, 2(5).

Lü, J.M. *et al.* (2010) 'Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems', *Journal of Cellular and Molecular Medicine*.

Mala, Sathiya, A.K. (2016). 'Nutritional Composition and Antioxidant Activity of Pumpkin Wastes', *International Journal of Pharmaceutical, Chemical and Biological Sciences*, 6(3): 336–344.

Mariita, R.M. *et al.* (2011) 'Methanol Extract of Three Medicinal Plants from Samburu in Northern Kenya Show Significant Antimycobacterial, Antibacterial and Antifungal Properties', *Research Journal of Medicinal Plant*, 5(1): 54–64.

Martínez Nieto, L. *et al.* (2009). 'Photodegradation of phytosanitary molecules present in virgin olive oil', *Journal of Photochemistry and Photobiology A: Chemistry*.

Saeed, N.; Khan, M.R. and Shabbir, M. (2012). 'Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L', *BMC Complementary and Alternative Medicine*. doi: 10.1186/1472-6882-12-221.

Santhosh Kumar, C.N.; Shridhar, N.B.; Jagadeesh, S.S.; Sanganal, J.S.; Suguna, R.; Ansar Kamran, C. (2014) 'Antioxidant activity of asteracantha longifolia in dmba induced mammary cancer in sprague dawley rats.', *Journal of Cell & Tissue Research*, 14(1): 4137–4141.

Valenzuela, G.M. *et al.* (2014) 'Evaluation Polyphenol Content and Antioxidant Activity in Extracts of Cucurbita spp', *Open Access Library Journal*, 1(03): 1–6.

Satyajit, D.; Zahid, L.; Alexander, I. (2012). Natural Products Isolation, Human press Totowa, New Jersey. Second ed, 2012, 20

Pascal, R. (1974). Plant phenolic, Oliver and Boyed. Edinboura, French Book, 254.

Amic, D.; Davidovic-Amic, D.; Beslo, D.; Rastija, V; Lucic, B.; Trinajstic, N (2007). SAR and QSAR of the antioxidant activity of flavonoids. Curr. Med. Chem., 14: 827–845

Harbone, J.B. (1984). Phytochemical methods agude to modentechniques of plant analysis. 2nd (edn).